

Carbohydrate Mobilization During Germination of Post-Diapausing Gemmules of the Freshwater Sponge *Eunapius fragilis*

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Abstract. Post-diapausing gemmules of the freshwater sponge *Eunapius fragilis* were found to contain sorbitol and glycogen as their primary carbohydrates. The sorbitol probably acts to increase the tolerance of the gemmules to freezing and desiccation. During germination, average sorbitol levels—measured as micromoles of sorbitol per gram of fresh weight of gemmule tissue ($\mu\text{mol/gfw}$)—declined from a control value of $36 \mu\text{mol/gfw}$ to about $4 \mu\text{mol/gfw}$. Concomitantly, average glycogen levels increased from a control value of $29 \mu\text{mol/gfw}$ to a steady-state level of $62 \mu\text{mol/gfw}$. It is probable that glycogen is being synthesized at the expense of sorbitol. The breakdown of sorbitol was associated with an increase in the activity of sorbitol dehydrogenase from undetectable levels in dormant gemmules to a maximum of $0.2 \mu\text{mol/min}\cdot\text{mg}$ protein after 30 h of exposure to 20°C . Aldose reductase activity remained constant throughout germination. These data support the hypothesis that the decrease in sorbitol levels is the result of an increase in the rate of catabolism by sorbitol dehydrogenase. The total activity of glycogen synthase did not change during germination; however, the activity of glucose-6-phosphate-dependent glycogen synthase was about 18 times greater than the activity of glucose-6-phosphate-independent glycogen synthase. Total glycogen phosphorylase activity increased from about $1.6 \text{ nmol/min}\cdot\text{mg}$ protein to $3.6 \text{ nmol/min}\cdot\text{mg}$ protein during germination. At the same time, however, the percentage of glycogen phosphorylase *a* decreased from almost 100% to about 84%.

This decrease would attenuate the apparent increase in activity. cAMP levels remained constant throughout germination. The observed changes in the level of glycogen in the gemmules are not simply due to changes in the activity of either glycogen phosphorylase or glycogen synthase.

Introduction

The life cycle of many freshwater sponges includes the formation of structures called gemmules. These consist of a mass of undifferentiated cells enclosed by a collagenous capsule. Gemmules are resistant to many environmental perturbations and are often produced as the sponge enters a period of environmental stress. For example, in southern New England the freshwater sponge *Eunapius fragilis* over-winters in the form of gemmules. After the sponge produces gemmules in the early fall, the adult tissue dies, leaving the gemmules attached to the substratum. In spring, the gemmules germinate to form the adult sponge again (Fell, 1987). In contrast, in temporary swamps in the southeastern United States the same species produces gemmules as the swamp dries in the summer. In this case the gemmules are exposed to hot, dry conditions and germinate when the swamps form again (Poirrier, 1969).

The physiological state of the gemmules from *Eunapius* changes with time and can be divided into three distinct stages: (1) diapause, (2) quiescence, and (3) germination (Fell, 1987). As gemmules are formed by *Eunapius fragilis*, they are initially in diapause, a state of dormancy controlled by some as yet unknown endoge-

nous factor or factors. In this state, the gemmules will not germinate even if exposed to favorable conditions. For example, gemmules that are collected in New England just after formation will not germinate when they are warmed to 20°C, even if kept at that temperature for up to 4 months. Quiescence occurs in gemmules collected at least 3 to 4 months after formation. In this case, the metabolism and development of gemmules is simply being depressed by low temperature, and they will readily germinate when warmed to 20°C. Similarly, in the laboratory, diapause can be broken by exposing the gemmules to cold (5°C) for 4 to 5 months or by exposing them to 5°C for 4 to 6 weeks followed by desiccation for 7 days (Fell, 1987). The breaking of diapause represents a basic shift from endogenous control of dormancy to environmental control of quiescence.

Germination of gemmules represents the period of development leading from post-diapause quiescence to the differentiated adult sponge. In *Eumapius*, emergence of the adult sponge tissue from the gemmule occurs 42 to 50 h after exposure to 20°C. Very little is known of the biochemical changes that occur during germination. Almost all of the information about these changes is based on nonquantitative histochemical methods and is limited to compounds easily stained in thin section (Simpson, 1984). The purpose of this paper, therefore, is to identify the major carbohydrates present in the post-diapausing gemmules of *Eumapius fragilis* and to examine changes in the levels of these compounds during germination.

Materials and Methods

Gemmules

Gemmules used for the measurement of possible energy sources during germination were collected from Gallop Pond in North Stonington, Connecticut, in late October of 1991. Gemmules used in the enzyme studies were collected from Stony Brook in South Hadley, Massachusetts, during early February of 1992. All gemmules were kept at 5°C until June to ensure that diapause was broken.

Germination experiments

Samples of gemmules ranging in size from 100 to 300 mg wet weight were placed in individual test tubes containing 10 ml of filtered stream water at 5°C. At the initiation of the experiment, the cold stream water was decanted from all of the gemmule samples and was replaced by filtered stream water at 20°C. All samples were well aerated and maintained at 20–23°C. Periodically, the stream water was decanted from three of the gem-

mule samples, which were then homogenized according to the protocol for the particular experiment (as outlined below).

Extraction of carbohydrates

Glycogen. Glycogen was extracted from the gemmules with cold 80% ethanol. Gemmules (300 mg) were ground under liquid nitrogen in a mortar and pestle. The powder was transferred to 50-ml centrifuge tubes and the liquid nitrogen was allowed to evaporate. Five milliliters of 80% ethanol was added to the centrifuge tubes containing the powdered gemmules and was mixed thoroughly on a vortex mixer. The ethanol solution was placed at 4°C overnight to allow all of the glycogen to precipitate and was then centrifuged at 10,000 × *g* for 20 min. The supernatant was retained for ethanol-soluble carbohydrate measurement and the pellet was washed twice more with 2 ml of cold 80% ethanol. The washings were combined with the original supernatant. The final pellet was dissolved in 5 ml of 30% KOH and heated to 100°C in a boiling water bath. After the sample was cooled to room temperature, 7.5 ml of 80% ethanol was added, and the glycogen was precipitated at 4°C overnight. Glycogen was collected by centrifuging the sample at 10,000 × *g* for 20 min and the resulting pellet was washed twice more with cold 80% ethanol. The pellet was dried under nitrogen, then dissolved in 5 ml distilled water; the amount of glycogen in the solution was measured using the anthrone method of Umbreit *et al.* (1972). The amount of glycogen measured using this method was not significantly different from that measured by the amyloglucosidase-based assay.

Identification of ethanol-soluble carbohydrates

High-performance liquid chromatography (HPLC). The combined supernatant from the ethanol extraction was dried under nitrogen, redissolved in either H₂O (for HPLC) or D₂O (for NMR) and filtered through a 0.45-μm filter prior to further analysis. Initial identification of carbohydrates in these samples was determined by HPLC using a Waters Radial-Pak silica cartridge modified with tetraethylenepentamine according to the methods of Hendrix *et al.* (1981). Carbohydrates were detected using an LDC/Milton Roy RefractoMonitor III.

Nuclear magnetic resonance (NMR). The ethanol-soluble carbohydrates were further characterized by ¹H- and ¹³C-NMR on a Bruker AC250 NMR. ¹H- and ¹³C-NMR spectra were measured at 250.1 and 62.9 MHz and chemical shift values are reported relative to the internal standard, sodium, 2,2-dimethyl-2-silapentane-5-sulfonate (DSS).

Sorbitol. Sorbitol in the ethanol-soluble fraction was

measured enzymatically using the method of Bergmeyer *et al.* (1974). The incubation solution contained 1 unit of sorbitol dehydrogenase and 1.2 mM NADH in 200 mM sodium phosphate buffer, pH 9.5, in a total volume of 2.0 ml. The amount of sorbitol was measured by incubating aliquots (50 μ l) of standards and samples in the incubation medium for 30 min and measuring the change in absorbance at 340 nm.

Enzyme assays

Sorbitol dehydrogenase. Sorbitol dehydrogenase activity was measured at 25°C in extracts of gemmules according to a modification of the method of Wolff (1955). Gemmules (100 mg) were homogenized in a Tenbroek homogenizer in 300 μ l of grinding buffer (20 mM Tris-HCl, pH 7.32). The homogenate was centrifuged at 10,000 $\times g$ for 10 min and an aliquot (100 μ l) of the supernatant was placed in 1.7 ml of assay buffer, resulting in a final concentration of 300 μ M NAD in 100 mM Tris-HCl, pH 9.0. A baseline rate was recorded, and the assay was then initiated by the addition of 200 μ l of 1.5 M sorbitol (150 mM final concentration). Activity was followed by measuring the rate of change in the absorbance at 340 nm.

Aldose reductase. Aldose reductase activity in extracts of gemmules was measured at 25°C using a modification of the method of Velle (1975). Gemmules were homogenized as described in the methods for sorbitol dehydrogenase and an aliquot (100 μ l) of the protein solution was placed in 1.6 ml of an assay buffer, resulting in a final concentration of 330 μ M NADPH in 300 mM Tris-HCl, pH 7.5. A baseline rate was recorded, and the assay was initiated by the addition of 300 μ l of 100 mM glyceraldehyde (45 mM). Activity was followed by measuring the rate of change in the absorbance at 340 nm. Aldose reductase has a broad substrate specificity and will catalyze the reduction of a number of aldoses (Hayman and Kinoshita, 1965). Glyceraldehyde (which results in the synthesis of glycerol) was used as a substrate in these assays instead of glucose (which results in the synthesis of sorbitol) to obtain increased sensitivity (Hayman and Kinoshita, 1965).

Glycogen synthase. Glycogen synthase activity was measured at 25°C in extracts of gemmules according to a modification of the methods of Mendicino *et al.* (1975). Gemmules (300 mg) were homogenized in 0.9 ml of 50 mM Tris-HCl, pH 7.5, containing 0.3 M sucrose, 20 mM 2-mercaptoethanol, and 5 mM EDTA. The homogenate was centrifuged at 10,000 $\times g$ for 10 min and an aliquot (100 μ l) of the supernatant was added to 0.21 ml of an assay buffer, resulting in a final concentration of 117 mM Tris-HCl, pH 7.5, 29 mM cysteine, and

100 mg/ml of glycogen. Glucose-6-phosphate-dependent activity was measured by the addition of glucose-6-phosphate to the assay solution to a final concentration of 11.8 mM. The assay was initiated by the addition of UDP-[¹⁴C]-glucose to a final concentration of 4.3 mM (specific radioactivity of 50,000 cpm/ μ mole). The assay was terminated after 30 min by the addition of 1 ml of 10% trichloroacetic acid. The resulting solution was centrifuged at 10,000 $\times g$ for 10 min. The supernatant was removed and added to 4 ml of 95% ethanol (-20°C) to precipitate the glycogen. The glycogen was pelleted by centrifuging at 10,000 $\times g$ for 10 min. The supernatant was discarded and the glycogen pellet was dissolved in 1 ml distilled water. The glycogen was reprecipitated twice more by adding 4 ml of 95% ethanol (-20°C) to this solution, followed by centrifugation. The amount of ¹⁴C incorporated into the glycogen from UDPG during the incubation period was measured in a liquid scintillation counter.

Glycogen phosphorylase. Glycogen phosphorylase activity was measured at 25°C in extracts from gemmules according to a modification of the method of Mendicino *et al.* (1975). Gemmules (100 mg) were homogenized in 300 μ l of 50 mM imidazole buffer (pH 7.0), containing 10 mM magnesium chloride, 2 mM EDTA, 0.01% bovine serum albumin, and 1% glycogen. The solution was centrifuged at 10,000 $\times g$ for 10 min and the supernatant was retained for the assay. Total activity was measured in 1 ml of 50 mM imidazole, pH 7.0, containing 10 mM magnesium chloride, 2 mM EDTA, 0.01% bovine serum albumin, 1.1% glycogen, 70 mM potassium phosphate, 300 μ M NADP, 10 μ M glucose-1,6-diphosphate, 4 mM AMP, 1 unit phosphoglucosylase, and 1 unit glucose-6-phosphate dehydrogenase. When phosphorylase *b* was measured, the AMP was omitted. The assay was initiated by the addition of 100 μ l of the protein solution. Activity was determined by measuring the rate of change in the absorbance at 340 nm. Protein concentration in extracts used for all enzyme assays was determined according to the methods of Peterson (1977).

cAMP assay. Adenosine 3',5' cyclic monophosphate (cAMP) in extracts from gemmules of *Eunapius fragilis* was measured using an ¹²⁵I radioimmunoassay according to the methods outlined in the Rianen (Dupont Medical Products) Assay System. Samples were prepared by homogenizing 30 mg of gemmules in 0.5 ml of cold (4°C) 6% trichloroacetic acid. cAMP recovery during extraction was determined by adding about 0.1 pmol (4,000 CPM) of ³H cAMP to the homogenate as an internal standard. The extracts were centrifuged at 2500 $\times g$ for 15 min, and the supernatant was extracted four times with 2.5 ml of water-saturated ether. The aqueous layers were then dried under a stream of air at 70°C. The

resulting residue was dissolved in 0.5 ml of the radioimmunoassay assay buffer, and cAMP concentrations were measured in 100- μ l aliquots of this solution.

Statistical analysis. Statistical differences among treatments were assessed by analysis of variance. Values are expressed as means \pm 1 SE.

Results

Ethanol-soluble carbohydrates

HPLC analysis of the ethanol-soluble fraction showed a single peak that co-eluted with sorbitol and remained single when spiked with authentic sorbitol (data not shown). Because it is difficult to exclude the presence of other six-carbon polyols solely on the retention times on

the HPLC column, we used ^1H - and ^{13}C -NMR, which together can distinguish the isomeric hexitols, to further analyze the ethanol-soluble fraction. The spectra for both ^1H - and ^{13}C -NMR were virtually identical to the spectra, obtained under identical acquisition conditions, for authentic sorbitol (Fig. 1). The chemical shifts for ^{13}C -NMR are shown in Table I. Further, there were no additional peaks in the spectra, eliminating the possibility of other compounds being present. Based on the HPLC and NMR data, it is clear that sorbitol comprises the major ethanol-soluble carbohydrate in post-diapause gemmules.

Sorbitol levels determined by sorbitol dehydrogenase and expressed as micromoles per gram of fresh gemmule tissue ($\mu\text{mol/gfw}$) declined rapidly in germinating gem-

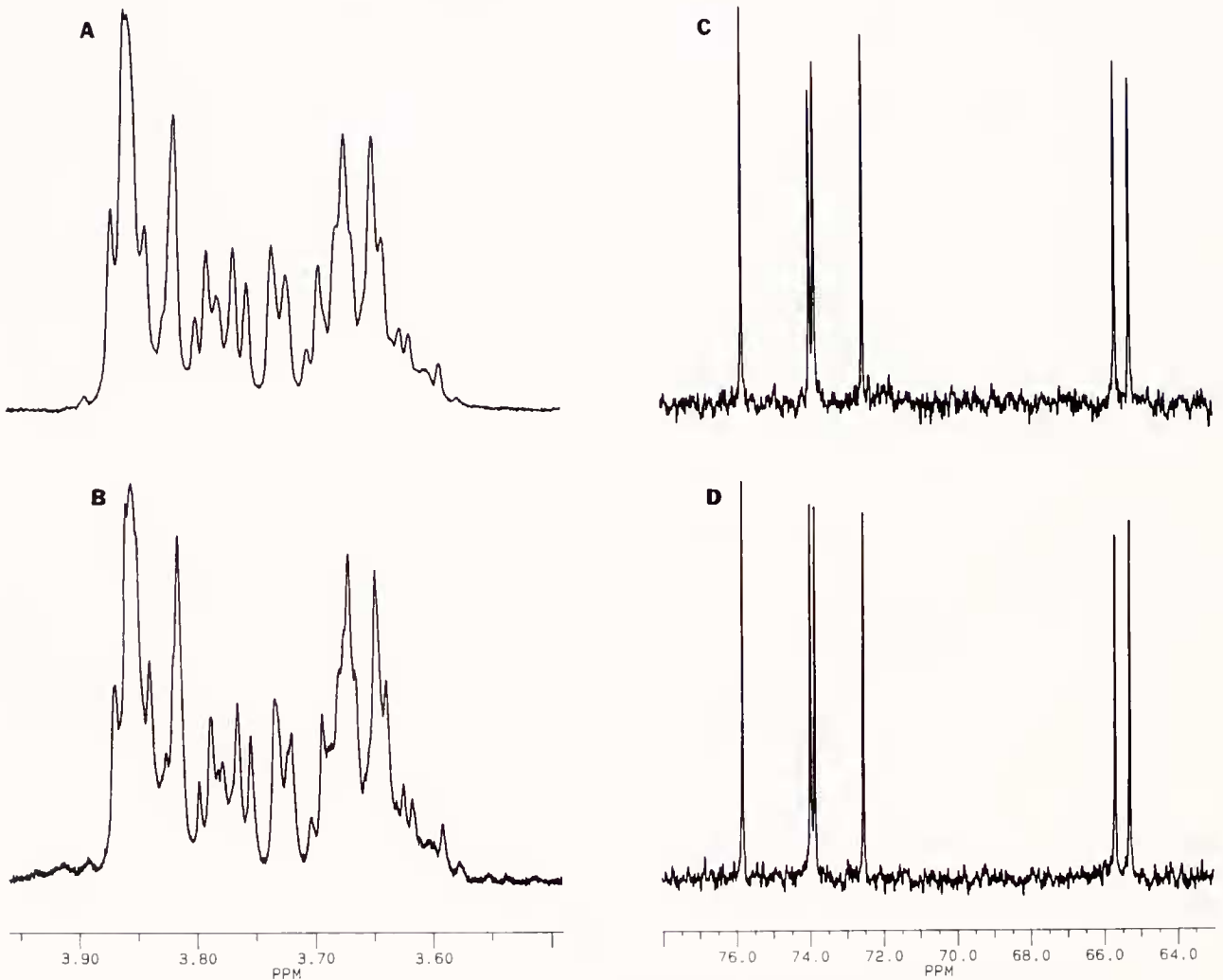


Figure 1. ^1H -NMR spectra of authentic sorbitol (A) and the ethanol-soluble fraction of *Eunapius fragilis* gemmules (B) and ^{13}C -NMR spectra of authentic sorbitol (C) and the ethanol-soluble fraction of *Eunapius fragilis* gemmules (D).

Table 1

¹³C-NMR chemical shifts measured downfield from the internal standard DSS in D₂O of authentic sorbitol and the ethanol soluble fraction from gemmules of *Eunapius fragilis*

Sample	¹³ C chemical shifts					
<i>E. fragilis</i> extract	65.3	65.7	72.5	73.8	74.0	75.8
Sorbitol	65.2	65.7	72.5	73.8	73.9	75.8

mules—from a maximum of about 36 $\mu\text{mol/gfw}$ to a steady-state level of about 4 $\mu\text{mol/gfw}$ by 36 h (Fig. 2). Sorbitol remained constant over the same time in gemmules maintained at 5°C. Concomitant with the decline in sorbitol, there was a significant increase in glycogen content measured by anthrone—from about 29 μmol hexose equivalents/gfw to about 62 μmol hexose equivalents/gfw (Fig. 3). As with the sorbitol, glycogen levels remained constant when gemmules were maintained at 5°C.

Enzyme activities

Sorbitol dehydrogenase activity was undetectable in the quiescent gemmules. During gemmule germination at 20°C, activity increased dramatically to a maximum of 0.2 $\mu\text{mol/min}\cdot\text{mg}$ protein at 36 h (Fig. 4). Between 36 and 48 h, the activity declined again, to about 0.05 $\mu\text{mol/min}\cdot\text{mg}$ protein. In contrast, the activity of aldose reductase remained at about 8 $\mu\text{mol/min}\cdot\text{mg}$ protein throughout germination (Fig. 4).

Total glycogen phosphorylase activity increased by a factor of 2.3 during germination—from a low of 1.6 to a high of 3.7 $\text{nmol/min}\cdot\text{mg}$ protein (Fig. 5). Maximal activity occurred by 42 h. The percentage of glycogen phosphorylase *a* declined from a maximum of 100% in quiescent gemmules to a minimum of 84% at 42 h. In contrast to changes in glycogen phosphorylase activity, there were no significant changes in the activity of either the glucose-6-phosphate-dependent or the glucose-6-phosphate-independent glycogen synthase during germination (Fig. 6). The activity of the glucose-6-phosphate-dependent glycogen synthase is 18 times greater than the activity of the glucose-6-phosphate-independent glycogen synthase. Note, however, that the total glycogen phosphorylase activity is three times higher than the glucose-6-phosphate-independent glycogen synthase activity and one-fourth of the activity of the glucose-6-phosphate-dependent glycogen synthase activity.

cAMP concentrations did not change significantly during the germination of gemmules of *Eunapius fragilis*, ranging from 3.6 ± 0.7 to 4.0 ± 0.8 pmol/mg protein.

Discussion

Germination of *Eunapius fragilis* gemmules under the conditions described here occurs during the first 42 to 50 h following exposure to 20–23°C. During that time, sorbitol levels decline dramatically and glycogen levels increase. It is possible that the sorbitol is being used to synthesize glycogen. Indeed, the changes in the two carbohydrates—measured as micromoles per gram of fresh

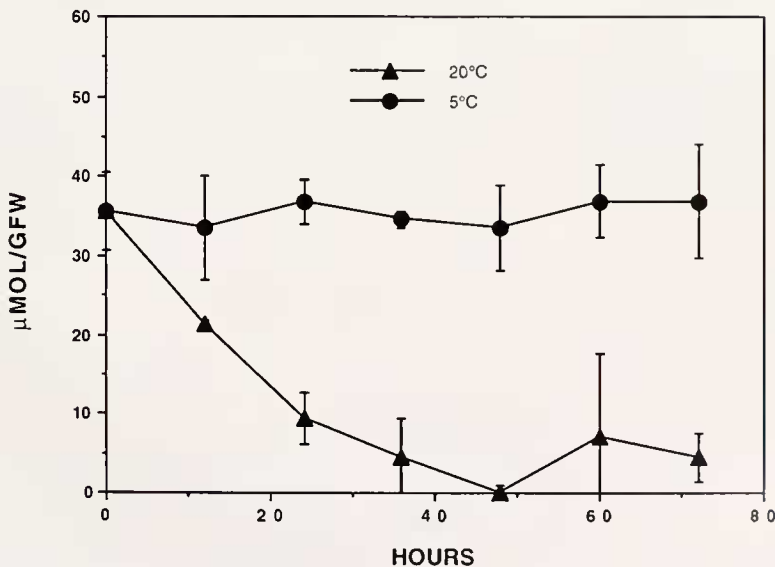


Figure 2. Changes in the levels of sorbitol in gemmules of *Eunapius fragilis* during germination. Values are expressed as means \pm 1 SE ($n = 3$).

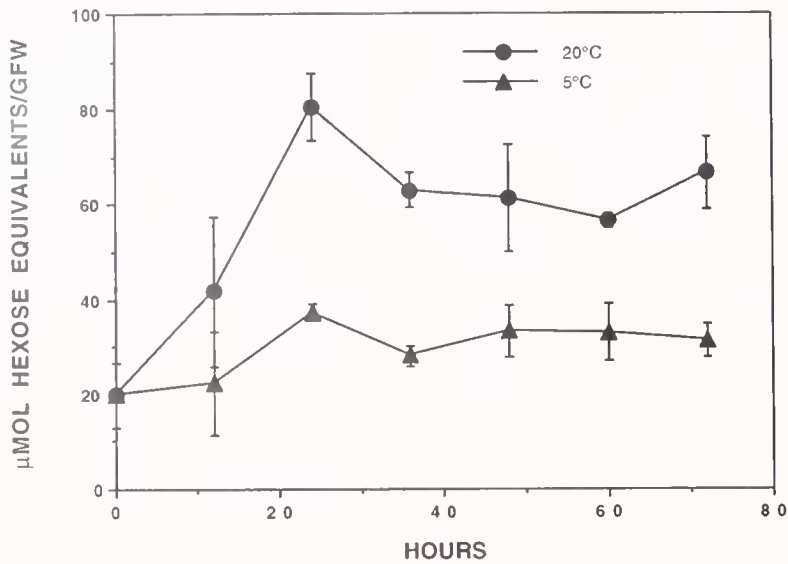


Figure 3. Changes in the levels of glycogen in gemmules of *Eunapius fragilis* during germination. Values are expressed as means \pm 1 SE ($n = 3$).

weight of gemmule tissue ($\mu\text{mol/gfw}$)—balance one another very closely: sorbitol decreases by $32 \mu\text{mol/gfw}$ (from an average control value of $36 \mu\text{mol/gfw}$ to an average steady-state level of $4 \mu\text{mol/gfw}$), and glycogen increases by $33 \mu\text{mol/gfw}$ (from an average control value of $29 \mu\text{mol/gfw}$ to an average steady-state value of $62 \mu\text{mol/gfw}$). The synthesis of glycogen from a carbohydrate precursor is consistent with observations for other develop-

ing invertebrates; for example, during the first few hours of germination, the osmotic pressure of gemmules of *Spongilla lacustris* drops precipitously (Rasmont, 1975). Rasmont (1975) attributed this reduction in osmotic pressure to the resynthesis of glycogen from an unidentified low molecular weight precursor. Also, in insect larvae, the synthesis of glycerol and sorbitol during exposure to cold occurs at the expense of glycogen, and

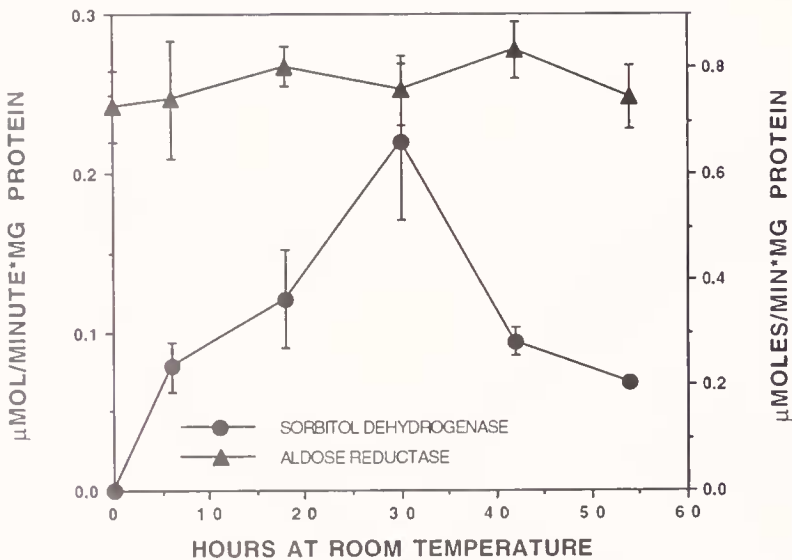


Figure 4. Changes in the activity of sorbitol dehydrogenase and aldose reductase in gemmules of *Eunapius fragilis* during germination. Assay conditions are as outlined in the Materials and Methods section. Values are expressed as means \pm 1 SE ($n = 3$).

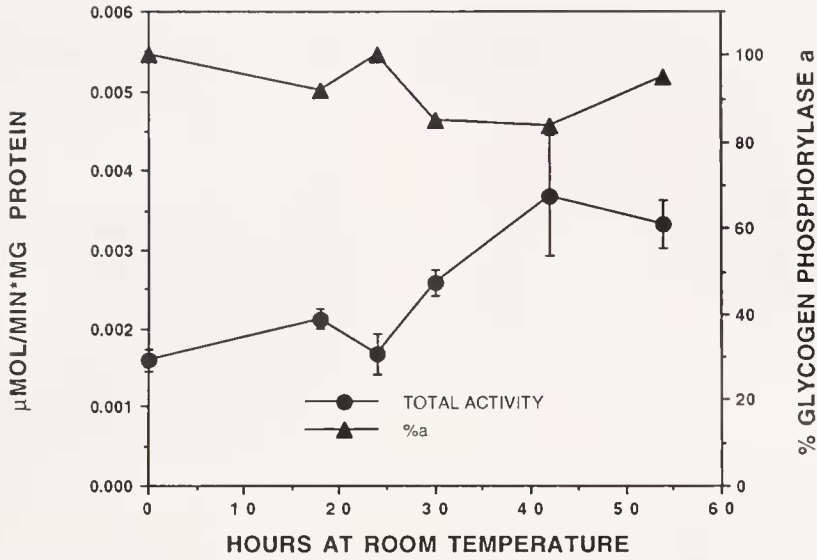


Figure 5. Changes in the activity of total glycogen phosphorylase and the percentage of glycogen phosphorylase *a* in gemmules of *Eunapius fragilis* during germination. Assay conditions are as outlined in the Materials and Methods section. Values are expressed as means \pm 1 SE ($n = 3$).

during subsequent warming, glycogen levels are subsequently restored at the expense of sorbitol (Storey and Storey, 1983). Clegg (1964) reports that during the pre-emergence development of *Artemia salina* cysts, trehalose is utilized to synthesize glycogen and glycerol during development. In this case, the decrease in trehalose can account for all of the synthesis of glycogen and glycerol plus the oxygen consumption measured during pre-

emergence (Clegg, 1964). This is not the case for *Eunapius fragilis*, however. The amount of sorbitol breakdown can just account for the amount of glycogen synthesized. Calorimetric measurement of heat dissipation of the gemmules of *Eunapius fragilis* shows that the energy flow of gemmules increases dramatically during germination (Loomis *et al.*, 1996). Clearly, another source of energy must be available during germination. This

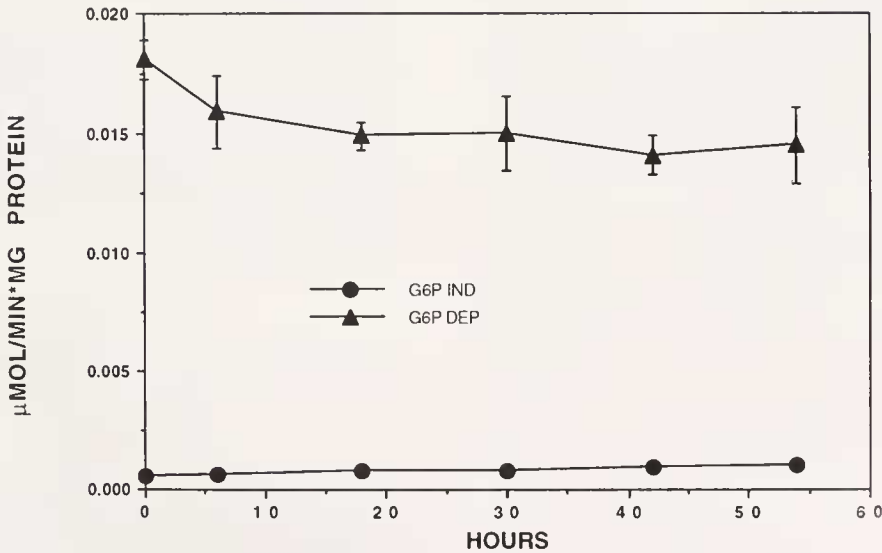


Figure 6. Changes in the activity of glucose-6-phosphate-dependent and glucose-6-phosphate-independent glycogen synthase in gemmules of *Eunapius fragilis* during germination. Assay conditions are as outlined in the Materials and Methods section. Values are expressed as means \pm 1 SE ($n = 3$).

source of energy is most probably nutrients contained in yolk platelets. The thesocytes of gemmules are packed with yolk platelets, and these platelets begin to shrink about 24 h after the initiation of germination in *Spongilla lacustris* (Simpson, 1984).

Using histological techniques, De Vos (1977) has shown that glycogen is present in the early stages of gemmule formation and later disappears. This is consistent with the lower levels of glycogen measured in post-diapausing gemmules of *Eumapius fragilis*. It may be that the glycogen originally present is used to synthesize sorbitol during development of gemmules, although this has yet to be determined.

Sorbitol is a well-known cryoprotectant produced during the winter in insects (see Lee, 1989; Baust *et al.*, 1985, for reviews), and it probably imparts increased freezing and desiccation tolerance to gemmules of *Eumapius fragilis*. In fact, gemmules of *Eumapius fragilis* are remarkably tolerant to freezing and desiccation (Fell, 1990; Boutselis *et al.*, 1990). For example, post-diapausing, fully hydrated gemmules will germinate following a 30-day exposure to -72°C (Boutselis *et al.*, 1990), and they survive air-drying for several weeks (Fell, 1990). Increased tolerance to cold and desiccation is no doubt important for the survival of *Eumapius fragilis* since this species is found in environments that can dry in the summer (such as swamps in the southeastern United States) and freeze in the winter (such as ponds and streams in the northeastern United States).

To begin to understand the biochemical mechanisms controlling the breakdown of sorbitol and the synthesis of glycogen during germination in *Eumapius fragilis* gemmules, we measured the activities of the proximate enzymes responsible for catabolism and anabolism of each of these compounds. Of course such analyses do not rule out modulations in other auxiliary enzymes that may also contribute to the altered mobilization of carbohydrate stores.

Sorbitol breakdown may result from an increase in the activity of sorbitol dehydrogenase (the catabolic enzyme), a decrease in the activity of aldose reductase (the anabolic enzyme), or both. In *Eumapius*, sorbitol breakdown during the germination of gemmules appears to be the result of an increase in the activity of sorbitol dehydrogenase. The activity of this enzyme increases from undetectable levels in quiescent gemmules to more than $0.2 \mu\text{mol}/\text{min} \cdot \text{mg}$ protein during the first 30 h of germination; during the same period, more than 95% of the sorbitol breakdown occurs. Aldose reductase activity remains unchanged throughout germination. Therefore, increased catabolic capacity could account for the observed changes in sorbitol levels during germination. The increase in maximal activity of the catabolic enzyme,

sorbitol dehydrogenase, could be the result of either *de novo* synthesis of the enzyme or catalytic activation *via* covalent modification during the early stages of germination; it is still unclear whether one or both of these processes are responsible.

Similarly, glycogen synthesis could result from an increase in the activity of glycogen synthase (the anabolic enzyme), a decrease in the activity of glycogen phosphorylase (the catabolic enzyme), or both. In *Eumapius*, there is no clear indication whether any of these possibilities occur. Glycogen synthase activity, whether dependent on or independent of glucose-6-phosphate, remains unchanged during germination. Total glycogen phosphorylase activity, on the other hand, increases during germination, but this increase is at least partly nullified by the conversion of some of the active glycogen phosphorylase *a* form to its inactive *b* form. Similarly, there are no changes in the concentration of cAMP (this paper), ATP or AMP (Loomis *et al.*, 1996) that could promote allosteric or covalent changes *in vivo* in glycogen phosphorylase and glycogen synthase. Indeed, the relatively high concentrations of cAMP may keep most of the glycogen synthase in the glucose-6-phosphate-dependent form. These data are in contrast with results from *Spongilla lacustris* gemmules in which cAMP levels are dramatically reduced during the first 2 h of exposure to 20°C (Simpson and Rodan, 1976). Simpson (1984) has suggested that the reduction is due to the combination of a decrease in adenylate cyclase activity and an increase in cAMP phosphodiesterase activity. In *Spongilla*, the low levels of cAMP could result in a decrease in the breakdown of glycogen through the inhibition of glycogen phosphorylase.

Clearly, other control mechanisms that do not involve changes in cAMP must be responsible for the synthesis of glycogen in gemmules of *Eumapius fragilis*. It is possible that changes in the concentration of glucose-6-phosphate during germination could increase the rate of glycogen synthesis by allosterically activating the glucose-6-phosphate-dependent glycogen synthase. We are examining this possibility.

Acknowledgments

This work was supported by NSF grants DCB-9018579 and IBN 9306652 to SCH.

Literature Cited

- Baust, J. G., R. R. Rojas, and M. D. Hamilton. 1985. Life at low temperatures: representative insect adaptations. *Cryo. Lett.* 6: 199–210.
- Bergmeyer, H. U., W. Gruber, and I. Gutman. 1974. D-Sorbitol. Pp. 1323–1326 in *Methods in Enzymatic Analysis*, H. U. Bergmeyer, ed. Academic Press, New York.
- Boutselis, N. J., P. E. Fell, and S. H. Loomis. 1990. Low tempera-

- ture tolerance of the gemmules of *Eunapius fragilis*. *J. Exp. Zool.* **255**: 125-129.
- Clegg, J. S. 1964.** The control of emergence and metabolism by external osmotic pressure and the role of free glycerol in developing cysts of *Artemia salina*. *J. Exp. Biol.* **41**: 879-892.
- De Vos, L. 1977.** Morphogenesis of the collagenous shell of the gemmules of a fresh-water sponge *Ephydatia fluviatilis*. *Arch. Biol.* **88**: 479-494.
- Fell, P. E. 1987.** Synergy between low temperature and desiccation in breaking gemmule diapause of *Eunapius fragilis* (Leidy). *Int. J. Invertebr. Reprod. Dev.* **12**: 331-340.
- Fell, P. E. 1990.** Environmental factors affecting dormancy in the freshwater sponge *Eunapius fragilis* (Leidy). *Invertebr. Reprod. Dev.* **18**: 213-219.
- Hayman, S., and J. H. Kinoshita. 1965.** Isolation and properties of lens aldose reductase. *J. Biol. Chem.* **240**: 877-882.
- Hendrix, D. L., R. E. Lee, J. G. Baust, and H. James. 1981.** Separation of carbohydrates and polyols by a radially compressed HPLC silica column modified with TEPA. *J. Chromatogr.* **20**: 45-53.
- Lee, R. E. 1989.** Insect cold-hardiness: to freeze or not to freeze. *Bio-science* **39**: 308-313.
- Loomis, S. H., S. C. Hand, and P. E. Fell. 1996.** Metabolism of gemmules from the freshwater sponge *Eunapius fragilis* during diapause and post-diapause. *Biol. Bull.* **191**: 385-392.
- Mendicino, J., H. Abou-Issa, R. Medicus, and N. Kratowich. 1975.** Fructose 1,6-diphosphate, phosphofructokinase, glycogen synthetase, phosphorylase, and protein kinase from swine kidney. *Methods Enzymol.* **42**: 375-392.
- Peterson, G. L. 1977.** A simplification of the protein assay method of Lowry *et al.* which is generally more applicable. *Anal. Biochem.* **83**: 346-356.
- Poirrier, M. A. 1969.** Louisiana fresh-water sponges: taxonomy, ecology, and distribution. Ph.D. thesis. Louisiana State University. 173 pp. Baton Rouge, LA.
- Rasmont, R. 1975.** Freshwater sponges as a material for the study of cell differentiation. *Curr. Top. Dev. Biol.* **10**: 141-159.
- Simpson, T. L. 1984.** *The Cell Biology of Sponges*. Springer-Verlag, New York. 622 pp.
- Simpson, T. L., and G. A. Rodan. 1976.** Role of cAMP in the release from dormancy of freshwater sponge gemmules. *Dev. Biol.* **49**: 544-547.
- Storey, J. M., and K. B. Storey. 1983.** Regulation of cryoprotectant metabolism in the overwintering gall fly larva, *Eurosta solidaginis*: temperature control of glycerol and sorbitol levels. *J. Comp. Physiol. B.* **149**: 495-502.
- Umbreit, W. W., R. H. Burris, and J. F. Stauffer. 1972.** *Manometric and Biochemical Techniques; a Manual Describing Methods Applicable to the Study of Tissue Metabolism*. Burgess Publishing Co., Minneapolis. 387 pp.
- Velle, W. 1975.** Aldose reductase from seminal vesicle and placenta of ruminants. *Methods Enzymol.* **41(B)**: 165-170.
- Wolff, John B. 1955.** Sorbitol dehydrogenase from liver. *Methods Enzymol.* **1**: 348-350.